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EVALUATION OF THE 10-DAY SEDIMENT TOXICITY TEST USING THE MIDGE (*CHIRONOMUS TENTANS*): THE EFFECT OF DIFFERENT WATER-SEDIMENT RATIOS ON BIOLOGICAL ENDPOINTS AND WATER QUALITY

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ABSTRACT

Midge larvae, *Chironomus tentans*, were tested in standard 10-day laboratory sediment toxicity tests using a number of contaminated freshwater sediments with varying chemical compositions and a range of concentrations. Tests were performed following two different experimental designs whose primary purpose was to evaluate the effect of sediment to water ratios on the biological response variables. The specific test procedures follow those described by Environment Canada and the Ontario Ministry of Environment and Energy. Concurrently, changes in the overlying water as measured by water quality parameters, were monitored throughout the test. Organism survival and growth results were independent of the sediment:water ratio and showed a similar degree in response among the respective sediment samples. Among the overlying water quality parameters, un-ionized ammonia concentrations exhibited the greatest degree of change between the two test procedures. This phenomena appeared to be related to the type of sediment tested and/or the volume of the test sample. Overall, the results suggest either test method provides reliable toxicity information for the assessment of sediment quality.

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1.0 INTRODUCTION

Whole-sediment toxicity testing has become a part of the regulatory framework for government agencies (ASTM 1995, Bedard *et al.*, 1992, EC 1992; 1995, US EPA 1994). Laboratory toxicity tests provide a cost-effective method for determining if sediment-associated contaminants are harmful to benthic organisms and aid in the remediation of contaminated sites (Jaagumagi and Persaud, 1996). There has been a recent attempt towards the standardization of freshwater sediment toxicity testing with benthic invertebrates within North America (Ingersoll *et al.*, 1995), to facilitate inter-laboratory comparability of test procedures and results. There is general agreement on the type of organisms found suitable for toxicity testing, response variables to be measured and the test duration (Burton, 1991). However, there appears to be less certainty regarding the acceptability of test conditions. Different approaches have been adopted or proposed within Ontario at the provincial and federal level for the midge sediment toxicity test. Both allow for a static system but differing water and sediment volumes.

The use of the midge in sediment toxicity tests has become universally recognized as a valid indicator organism for sediment quality (Burton, 1991; Giesy and Hoke, 1989). Detailed studies have been carried out to optimize the bioassay procedure. Ankley *et al.*, (1993) examined the effect of flow conditions, feeding rate and food type on water quality and test response using *C. tentans* in 10-day exposures. Similar work has been carried out using *C. riparius* (R. Scroggins, Environment Canada, *pers. comm.*). The effect of these test variables on organism lethality and growth have generally been tested on a limited number of contaminated field sediments or on relatively clean, inert substrates with an emphasis on either flow-through or static-renewal systems.

The objective of this study was to determine the degree of compatibility in terms of biological endpoints and secondarily on water quality parameters between the acute static tests using the midge larvae, *Chironomus tentans* according to the provincial and federal toxicity test protocols (EC 1995, Bedard *et al.*, 1992). Specifically, the study assessed the effect of different water to sediment ratios on midge survival and growth (measured as final weight). Another objective was to compare any subsequent changes in water quality parameters e.g. dissolved oxygen, conductivity, pH and ammonia, that would occur using the two test regimes (1:1.7 and 1:4; sediment:water ratio). This potential water quality change becomes more critical since the tests are performed under static conditions. The test was conducted using one clean, control sediment and five contaminated test sediments that resulted in a range of lethal and sublethal effects and distinct water quality profiles according to previous laboratory tests in 1995 (D. Bedard, MOEE, unpublished data). The selection of test sediments is important in order to assess the performance of the two test methods under a variety of toxicity scenarios.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Site Description

Sediment collected from Honey Harbour in Georgian Bay, Ontario, served as a control for each bioassay (Bedard *et al.*, 1992). The control sediment is a relatively uncontaminated sediment that provides a measure of test acceptability including control mortality criteria and the suitability of test conditions (ASTM, 1995). The control sediment will also act as a basis for comparing the biological responses from the test sediments.

The treatments consisted of surficial sediment that was collected from three different areas within the province in September 1995. The sediments were previously tested according to standard toxicity testing procedures (Bedard *et al.*, 1992). Collection was done using either an Ekman or Ponar grab sampler. At each sample location, approximately 10 L of composited surficial sediment (top 5 to 10 cm) was collected from several grabs. The composited sediment was placed into 20 L plastic buckets lined with food-grade polyethylene bags and transported to the Toronto, Ontario laboratory where they were stored at 4°C for at least 3 months prior to testing.

The following is a detailed description of the test sediments:

Treatment	Station Location and Sediment Description	Target Compound [a]
SED # 1	Collected from a stormwater pond near Cornwall, Ontario. Comprised mainly of light red clay, low amount of detritus, oligochaetes present.	None. Inorganic and organic compounds < SEL concentrations.
SED # 2	Located in the St. Marys River near Belleview Marine Park. Very high organic content, flocculent material. Oily sheen visible.	Oils and grease, total PAHs > LEL concentrations, some heavy metals.
SED # 3	Located in the St. Marys River near Belleview Marine Park. Organic enriched with moderate oily odour.	Oils and grease, total PAHs > LEL concentrations.
SED # 4	Sampled in Thunder Bay adjacent to the Northern Wood Preservers dock. Oily, dark grey/black sediment.	High total PAHs concentration.
SED # 5	Sampled in Thunder Bay adjacent to the Northern Wood Preservers dock. Oily, dark grey/black sediment.	High total PAHs concentrations.

[a] SEL and LEL refer to Severe and Lowest Effect Level concentrations as outlined in the Provincial Sediment Quality Guidelines (Persaud *et al.*, 1992). SEL represents a chemical concentration likely to be detrimental to 95% of benthic species and LEL likely to adversely effect 5% of benthic species.

2.2 Laboratory Biological Testing Methods

Chironomus tentans Culturing Procedure

The tests used 10-12 day old, cultured chironomid larvae with an average wet weight less than 1 mg. Approximately 12 egg masses were hatched to yield sufficient number of organisms to conduct the toxicity test and reference toxicant test. The MOEE continuously cultures *C. tentans* larvae from egg to adult following standard methods (Bedard *et al.*, 1992, Mosher *et al.*, 1987, Townsend *et al.*, 1981). Egg masses were acquired from Dr. J. Giesy at Michigan State University, Lansing, Michigan and have been cultured for several generations in our laboratory.

Initially, the midges were reared in enamelled trays for a period of 10 to 12 days and then maintained in a 21 L aquarium containing 1.2 L of fine silica sand and 0.4 L of medium-size silica sand. The cultures were held at room temperature (22 ± 1°C) with continuous aeration and under a 16:8 hour, light:dark photoperiod. The larvae were provided a vegetable diet *ad libitum*.

Chironomus tentans 10-Day Static General Test Procedure

Moist field-collected bottom sediment was pressed through a 2-mm stainless-steel sieve to remove existing large biota and debris prior to use. The sieved sediment was homogenized with a spatula and stored in 4 L acid-rinsed glass jars until required.

Second and third instar larvae were directly transferred from the enamelled rearing pans into the prepared test chamber (as per specific test method) using the 5-mm opening of a Pasteur pipette. The chambers were randomly placed into a holding tank at ambient room temperature and maintained under a 16:8 hour, light:dark photoperiod and continuous aeration.

Water in the exposure chambers was regularly monitored for pH, conductivity, total ammonia, un-ionized ammonia and dissolved oxygen. Parameters were checked on Day 1, 5 and 9 on a single test chamber/treatment. To measure ammonia in the 1:1.7 test method, 20 mL of overlying water was pooled from each of the five replicates. Water samples were carefully removed and replaced using a pipettor with the tip held against the side of the beaker. Any signs of abnormal behaviour of the test organisms or changes in appearance of the test chambers were noted. Water loss due to evaporation was replenished as needed.

After 10 days, the contents of the test chambers were emptied and washed in a sieve bucket. Surviving animals were sorted, removed and placed into 150 mL beakers holding 100 mL dechlorinated water and 15 mL silica sand. The larvae were counted, blotted dry and individuals weighed to the nearest 0.01 mg. For the 1:1.75 test method, the pooled animals for each replicate were transferred into aluminum weighing boats that were previously oven dried for 48 hours and pre-weighed. Dry weight determinations for the pooled midges were obtained after the samples were oven dried at 60°C for 24 hours, held in a desiccator and weighed to the nearest 0.01 mg. Empty weigh boats were also treated in a similar manner and recorded an error of 0.01%.

Specific Test Method for the 1:1.75 Beaker Test

Detailed test conditions are specified in Table 1. Sediment biological tests were conducted according to proposed Environment Canada standardized procedures with the following slight modification (EC, 1995). The experimental unit was a 250 mL glass beaker containing prepared sediment and dechlorinated municipal tap water (1:1.75 v:v). The test method recommends using a 300 mL high-form beaker. The 250 mL beaker was used as a substitute due to the unavailability of the 300 mL beaker. Environment Canada has previously conducted sediment toxicity tests using the 250 mL beaker with a 1:1 sediment:water ratio (Hamr *et al.*, 1994).

Ninety millilitre aliquots of homogenized sediment were placed into the test chamber and overlaid with 150 mL test water. A small piece of clean polyethylene was held 1 cm above the sediment surface while the water was slowly added in order to reduce sediment resuspension. Aeration of the overlying water commenced the same day that the test chambers were assembled. If control mortality exceeded 30%, the test is declared invalid.

The next day, a total of 10 animals were added per chamber to each of the five replicates. Animals were fed 15 mg of a slurry of Nutrafin Staple food flakes (0.525 g in 70 mL water) on Days 1, 3, 6 and 8, in each test chamber.

Specific Test Method for the 1:4 Jar Test

Table 1 provides detailed test conditions. Sediment biological tests were conducted according to MOEE standardized procedures (Bedard *et al.*, 1992) and are briefly described below. The experimental unit was a 1.8 L test chamber containing prepared sediment and dechlorinated municipal tap water (1:4, v:v).

Aliquots of homogenized sediment (325 mL) were placed into the test chamber and overlaid with 1.3 L test water. After settling overnight, the chambers were aerated continuously until the termination of the test. If control mortality exceeded 25%, the test is declared invalid.

The next day, a total of 15 animals were added per chamber to each of the four replicates. Animals were fed alternate days 30 mg of a Cerophyll®:Tetra Conditioning Vegetable® (3:2, w:w) diet.

Reference Toxicant Testing

A water-only reference toxicity ($CuSO_4$) test was conducted for 48-hours and LC50s were calculated. The static tests consisted of four test concentrations and a control. The

Table 1. Summary of Test Conditions for the Comparative Sediment Test using the Midge, *Chironomus tentans*.

Condition	1:1.75[a] Sediment:Water	1:4[b] Sediment:Water
Test Type	Static	Static
Sediment Volume	90 mL	325 mL
Overlying Water Volume	150 mL	1300 mL
Test Chamber Size	250 mL Beaker	1800 mL Jar
Surface Area	43 cm^2	130 cm^2
Temperature	22 (0.6)°C	22 (0.6)°C
Photoperiod	16L:8D	16L:8D
Age of Organisms	10 to 12 day	10 to 12 day
# of Organisms/chamber	10	15
# of Organisms/treatment	5	4
Food Type	Nutrafin Staple Food	Cerophyll/Tetra Cond.
Feeding Rate/chamber	15 mg / 4 X	30 mg / 7 X
Parameter Measurements	DO/pH/conductivity/ ammonia	DO/pH/conductivity/ ammonia
Frequency of Feeding	Days 1, 5 and 9	Days 1, 5 and 9
Test Duration	10 days	10 days
Endpoint	Survival and growth ind. wet and dry wts	Survival and growth ind. wet wts
Test Acceptability	>70% control survival	>75% control survival

[a] Environment Canada (1995)
[b] Bedard *et al.*, (1992)

nominal copper concentrations were 0.05, 0.25, 0.5, 1 and 3 mg/L. Ten midge larvae (10-12 day post-hatch, < 1 mg wet weight) were placed into each of four replicate 250 mL beakers. To help reduce stress, a fine layer of silica sand was added to the test containers. Mortality was monitored every 24 hours and water quality parameters were taken at 0 and 48 hours on one test chamber at each Cu concentration.

2.3 Statistical Methods

Statistical analyses were performed using SAS® software package (SAS, 1985). For each test, comparisons were made among the test and control sediments using One-Way Analysis of Variance (ANOVA) and Tukey's studentized range test (HSD) and planned comparisons (Steel and Torrie, 1960). Dunnett's *t*-test was used solely to compare mortality between the control and test sediments. Analysis was made on arc-sine transformed mortality data. Homogeneity of variance across groups was tested using Bartlett's test. Coefficients of variation (C.V.%) were calculated for each test response. In addition, two-Way Analysis of Variance (ANOVA) followed by Bonferroni's t-test was used to compare the various endpoints between the two test methods. LC50's (including the associated 95% confidence limits) were calculated using software developed by Stephan (1977) and were derived by probit analysis.

3.0 RESULTS

3.1 Reference Toxicity Test

The 48-hour copper LC50 (95% C.I.) for the water-only reference toxicant exposure for *C. tentans* was 0.82 (0.71 - 0.97) mg/L. This value was within the acceptable 48-h LC50 (± 2 s.d.) range of 1.44 (0.99) mg/L, according to a previous series of reference toxicant tests performed in our laboratory.

3.2 Effects of Sediment on Chironomid Lethality and Growth and Changes in Water Quality Parameters in the 1:1.75 Test Method

Conductivity, pH, total ammonia, un-ionized ammonia and dissolved oxygen parameters were periodically measured on the overlying water for single samples for each treatment and recorded in Table 2. Values are reported as mean ± standard deviation for the combined three time intervals. The pH and DO measurements were fairly stable among the test sites, pH ranged from 7.5 to 8.4 and DO from 7.7 to 8.3 mg/L. Average conductivity readings for each of the test sediments, with the exception of Sed #1, were comparable to the control reading of 358 umho/cm. On Day 1, the average conductivity among sediment types (Sed #1 omitted), was 293 (± 24) and increased to an average reading of 347 (± 36) umho/cm by Day 9 (Figure 1).

The amount of un-ionized ammonia in the overlying waters fluctuated among the majority of the test sediments over a concentration range of 0.05 to 0.50 mg/L (Figure 2).

TABLE 2. Mean (± standard deviation) water quality characteristics in sediment bioassays.

Parameter	pH		D.O. mg/L		Conductivity umho/cm		Total Ammonia mg/L		Un-ionized Ammonia mg/L	
Exposure Ratio	1:1.75	1:4	1:1.75	1:4	1:1.75	1:4	1:1.75[a]	1:4	1:1.75	1:4
Treatment										
Control	7.54 (.31)	8.01 (.08)	7.9 (0.4)	8.4 (0.1)	358 (26)	332 (12)	0.16 (0.05)	<0.10 (0.00)	0.003	0.004
SED #1	8.46 (.07)	8.48 (.07)	8.3 (0.2)	8.3 (0.0)	774 (99)	565 (82)	0.31 (0.12)	0.13 (0.05)	0.038	0.015
SED #2	8.07 (.21)	7.93 (.14)	8.0 (0.1)	8.0 (0.3)	302 (24)	295 (11)	4.06 (1.49)	1.53 (0.37)	0.254	0.059
SED #3	8.11 (.28)	8.10 (.18)	7.8 (0.3)	8.3 (0.3)	334 (25)	305 (7)	3.80 (2.13)	1.53 (0.50)	0.305	0.090
SED #4	7.87 (.03)	7.63 (.47)	7.7 (0.6)	7.0 (1.5)	305 (32)	301 (14)	2.26 (1.55)	0.92 (0.68)	0.087	0.028
SED #5	8.08 (.14)	7.96 (.20)	8.0 (0.1)	8.3 (0.5)	322 (59)	285 (11)	3.30 (0.26)	1.40 (0.52)	0.191	0.058

Sample size N=3 unless otherwise indicated

[a] Based on a pooled sample from 5 replicates.

Figure 1: Conductivity in 1:1.75 Exposure

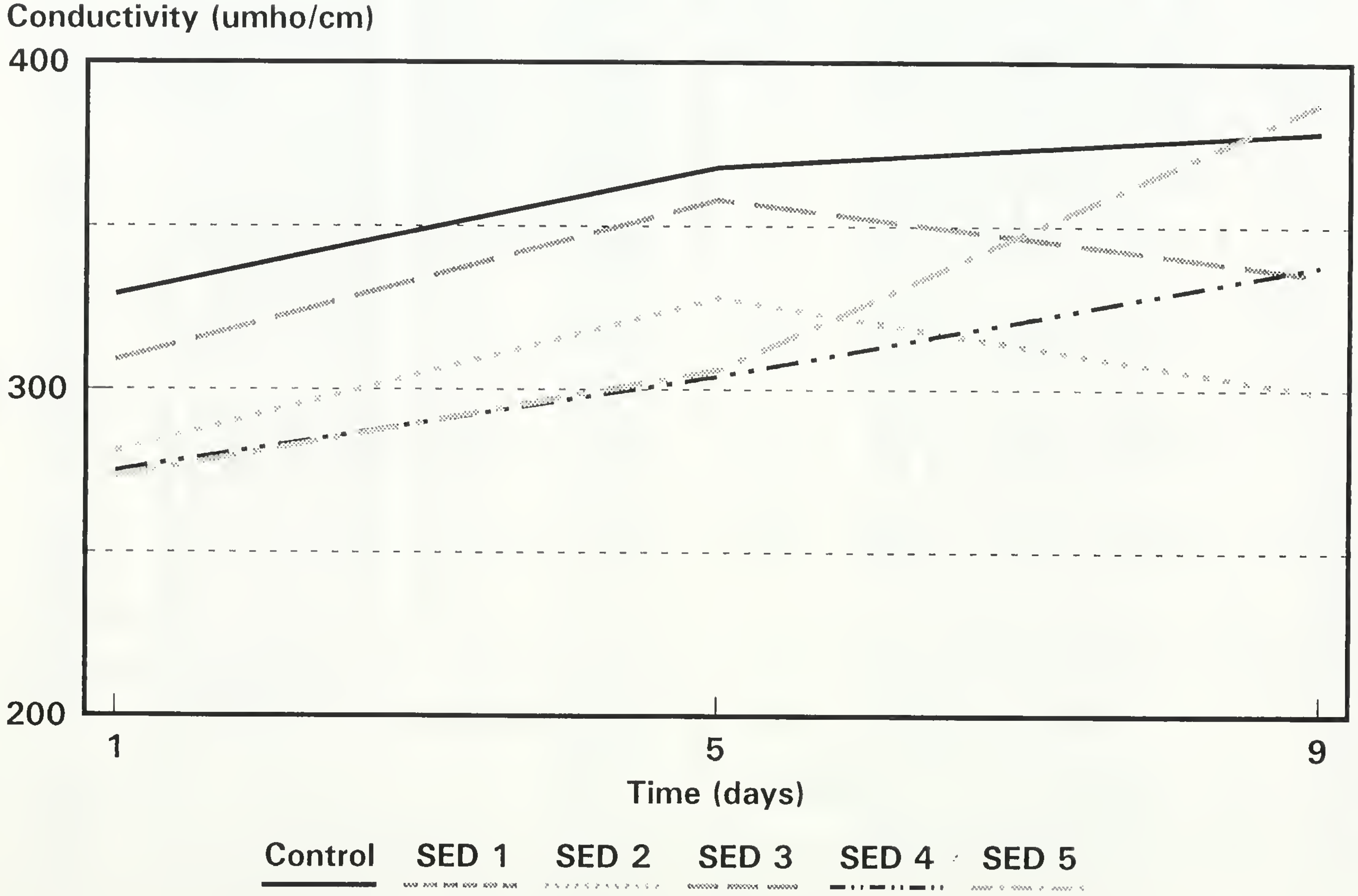
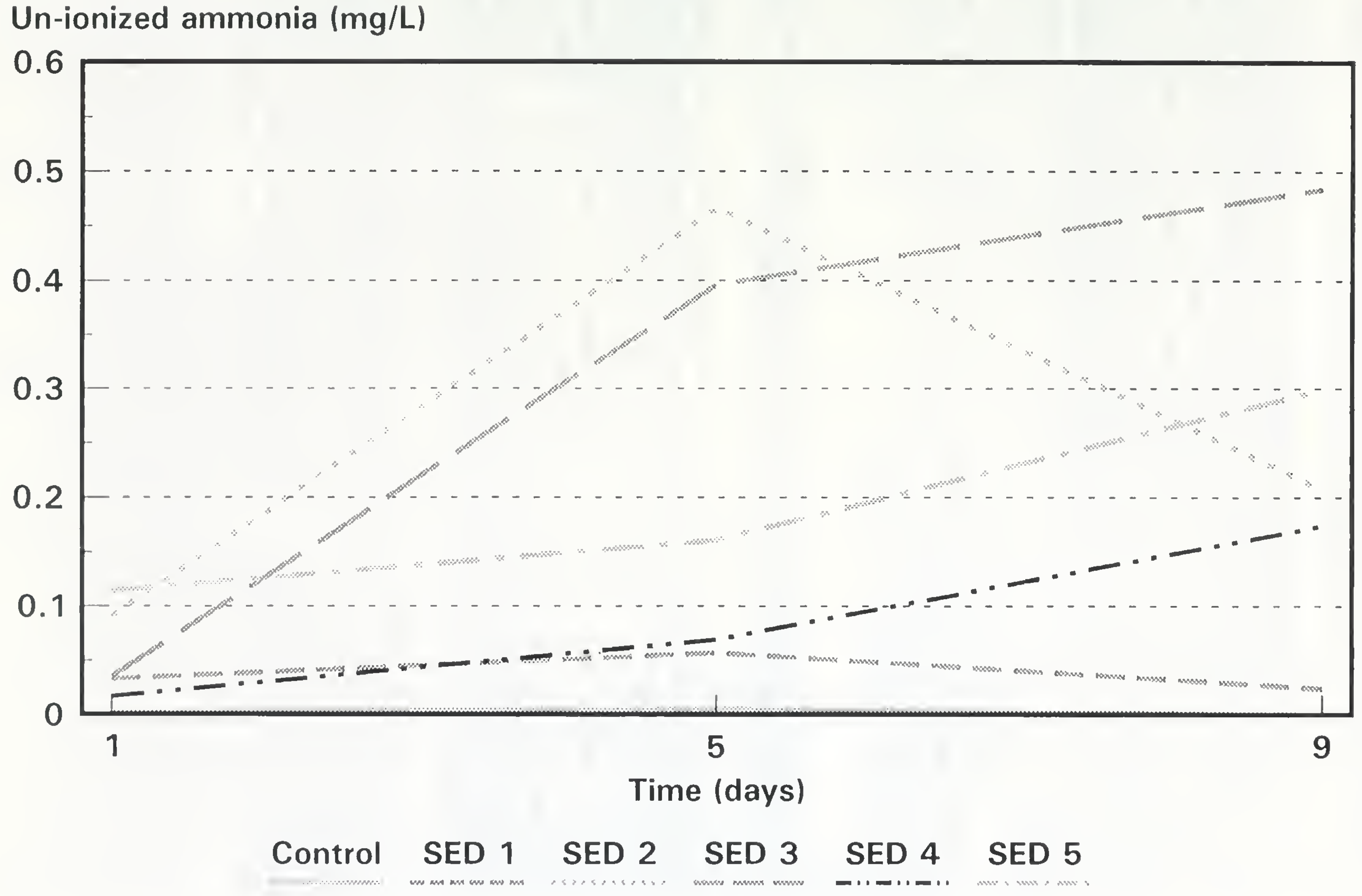


Figure 2: Un-ionized ammonia in 1:1.75 Exposure



A change in values between Day 1 and Day 9 indicated a positive increase of 126% to 1282% for Sediments #2, #3, #4 and #5. Among all treatments, the average initial reading was 0.04 mg/L and the final average reading was 0.19 mg/L.

The biological data for the two endpoints, mortality and growth, are summarized in Table 3 and depicted in Figures 3 and 4. Midge mortality was statistically higher for Sed #4 as compared to the control and other test sediments (100% mortality) ($p<0.0001$). A moderate degree of toxicity was noted for Sed #1 relative to the control (Dunnett's *t*-test; $p<0.05$) but received a similar ranking using Tukey's multiple range analysis. The remaining treatments elicited percent mortality values ranging from 4% to 28%. Significant differences in the sublethal growth endpoint was measured among sites ($p<0.0001$). The poorest growth occurred for Sed #2 (2.6 mg, wet weight), followed by Sed #3 (6.0 mg) and Sed #5 (7.1 mg). Each of these treatments had body weights significantly lower than the control animals of 10.3 mg.

As an alternative approach for measuring organisms growth, dry weight determinations were also made for each replicate. Multiple-range test among means indicated virtually identical ranking as reported on a wet weight basis (Table 3). Among the six treatments, the control animals received a lower ranking.

3.3 Effects of Sediment on Chironomid Lethality and Growth and Changes in Water Quality Parameters in the 1:4 Test Method

Conductivity, pH, total ammonia, un-ionized ammonia and dissolved oxygen parameters were periodically measured on the overlying water for single samples and recorded in Table 2. Values are reported as mean ± standard deviation based on measurements made over the course of the experiment. Both pH and dissolved oxygen values did not alter markedly during the test for each treatment. Dissolved oxygen within the test jars remained above acceptable levels (>4 mg/L) throughout the test (Range: 7.0 - 8.4) (OMOE, 1984). Similar pH measurements were recorded among the test sites (Range: 7.6 - 8.4). Conductivity of the overlying water remained stable throughout the duration of the test (Figure 5), except for Sed #1. Based on the other treatments, the average starting value was 305 (± 10) umho/cm and at the end of the test it held at 302 (± 28) umho/cm.

The trend in un-ionized ammonia readings was similar among the test sediments. Levels at the beginning of the test averaged 0.02 mg/L, peaked on Day 5 at 0.05 mg/L and dropped slightly to 0.04 mg/L by Day 9 (Figure 6). Two of the six sediments yielded a change in un-ionized ammonia readings of > 50%. This occurred for Sed #3 (142%) and Sed #5 (268%). Un-ionized ammonia in the overlying water remained within a narrow range of values, 0.05 mg/L to 0.14 mg/L, independent of sediment type or time interval.

Results for chironomid growth and lethality is reported in Table 3 and depicted in Figures 5 and 6. Percent mortality for the midge ranged from 8% to 96% There were significant differences in midge mortality among sites ($p<0.001$). Sediment #4 was highly toxic and was significantly higher than the control sediment (Dunnett's *t*-test, $p<0.05$). *Chironomus* growth varied significantly among treatments ($p<0.001$). Growth reported for

TABLE 3. Summary of biological results on midge sediment bioassays for control and test sediments. Mean values (± standard deviation).

Parameter	Percent Mortality		Average Individual Body Weight		
			(mg wet wt.)	(mg wet wt.)	(mg dry wt.)
Exposure Ratio	1:1.75	1:4	1:1.75	1:4	1:1.75
Treatment					
Control	ABC 14.0 (15)	A 8.3 (12)	A 10.30 (2.1)	A 10.30 (1.4)	B 1.40 (0.33)
SED #1	C* 42.0 (24)	A 29.9 (16)	A 11.38 (1.1)	A 11.58 (3.1)	A 2.23 (0.25)
SED #2	AB 12.0 (18)	A 29.9 (21)	C 2.67 (1.3)	C 2.05 (0.2)	C 0.43 (0.18)
SED #3	A 4.0 (9)	A 13.3 (11)	B 6.08 (0.6)	B 5.43 (1.3)	B 0.99 (0.08)
SED #4	D* 100 (0)	B* 96.6 (4)	–	–	–
SED #5	BC 28.0 (8)	A 26.6 (11)	B 7.18 (2.2)	B 5.39 (1.4)	B 1.25 (0.43)
Mean	33.3	34.1	7.52	6.95	1.26
Standard Deviation	12.3	12.4	1.47	1.49	0.25
% C.V.	36.9 %	36.3 %	19.5 %	21.4 %	19.8 %

A Means sharing a common letter within a column are not significantly different; Tukey's HSD test for % Mortality ($p<0.005$) and planned comparisons using LSMEANS for comparing Body Weight ($p<0.01$).

* % Mortality significantly different than the control; Dunnett's t–test, $p<0.05$).

Figure 3:Chironomid mortality for 1:1.75 and 1:4 Sediment to Water Ratio Exposures

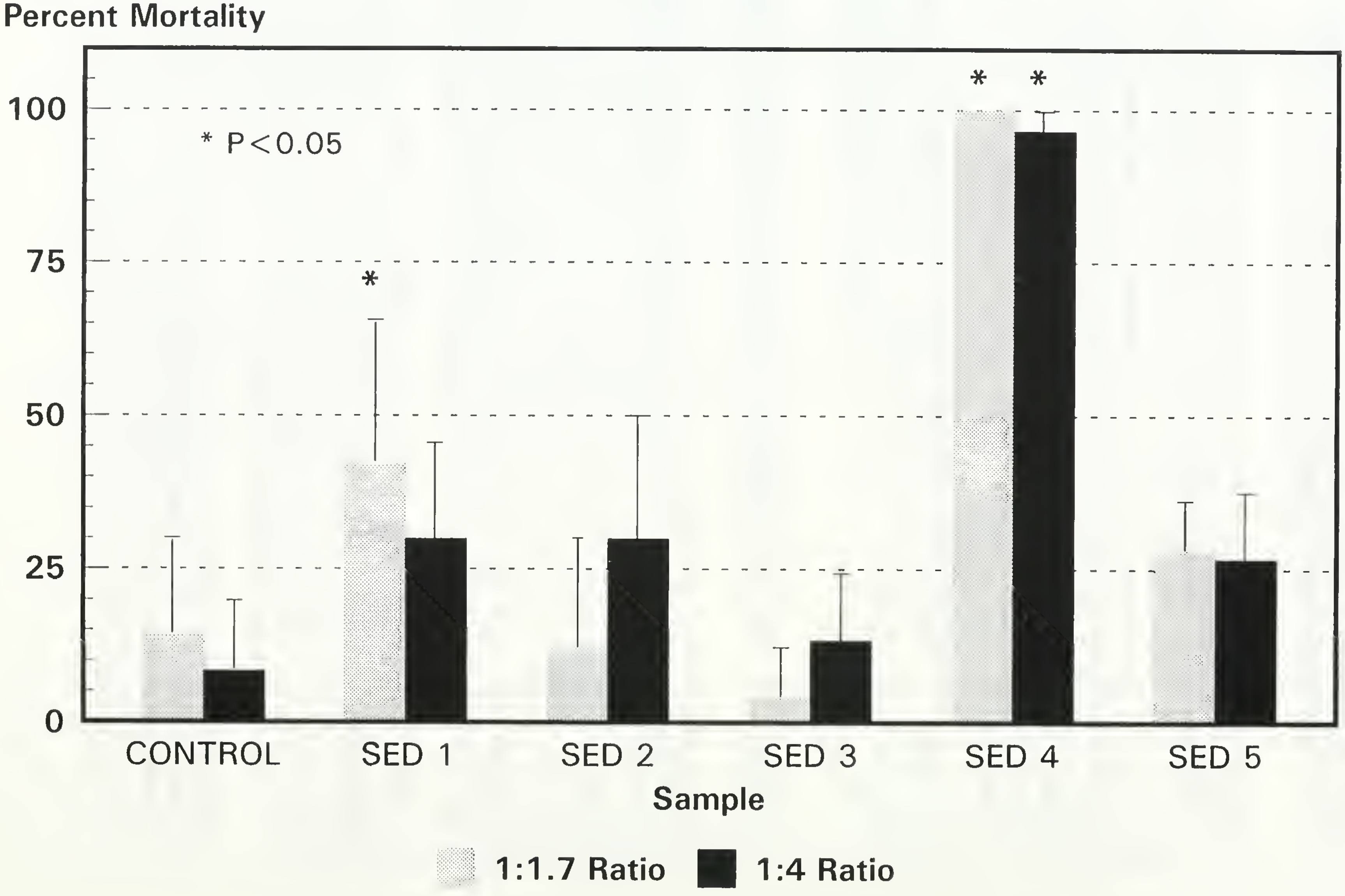


Figure 4: Chironomid growth for 1:1.75 and 1:4 Sediment to Water Ratio Exposures

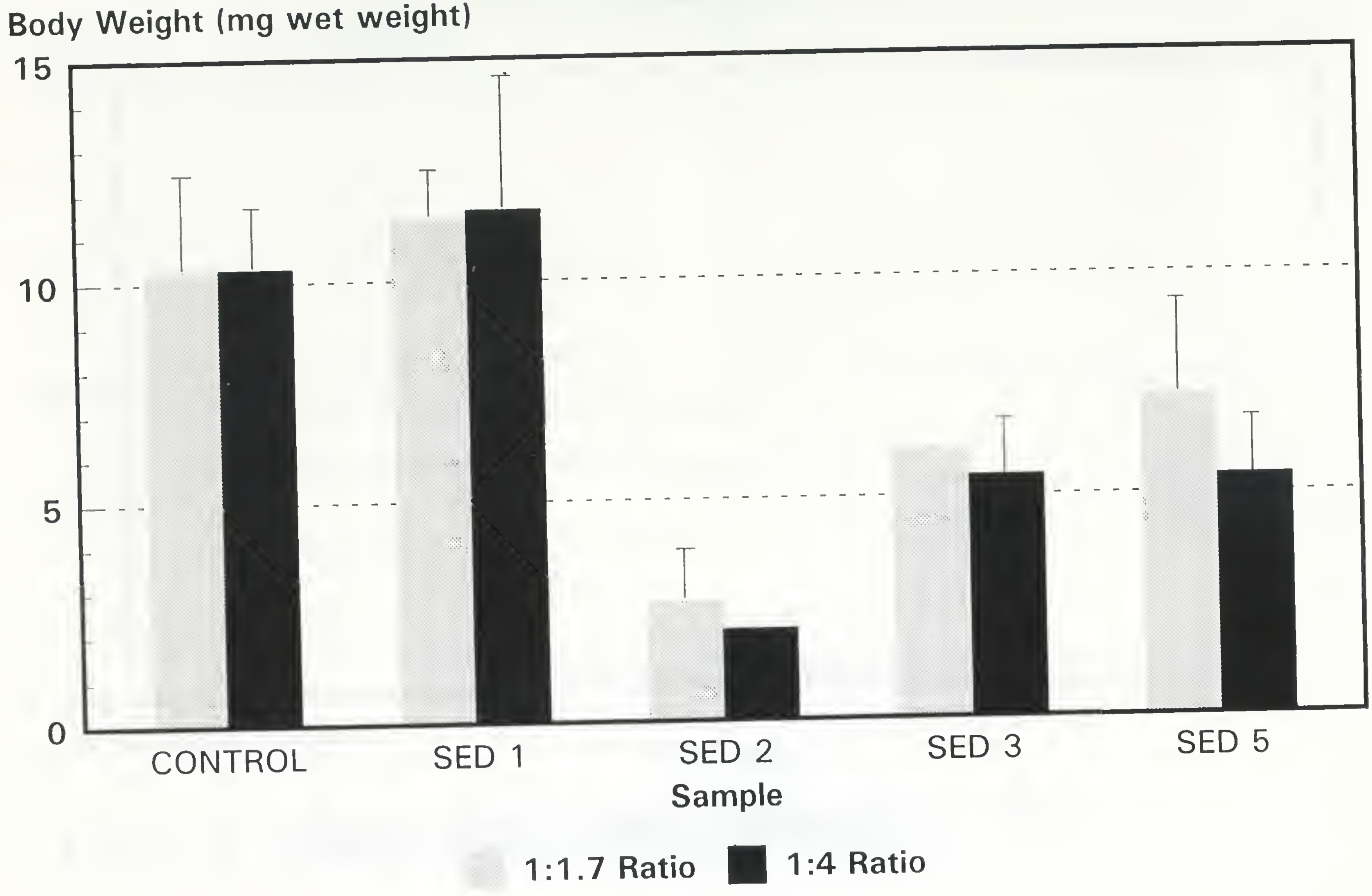


Figure 5: Conductivity in 1:4 Exposure

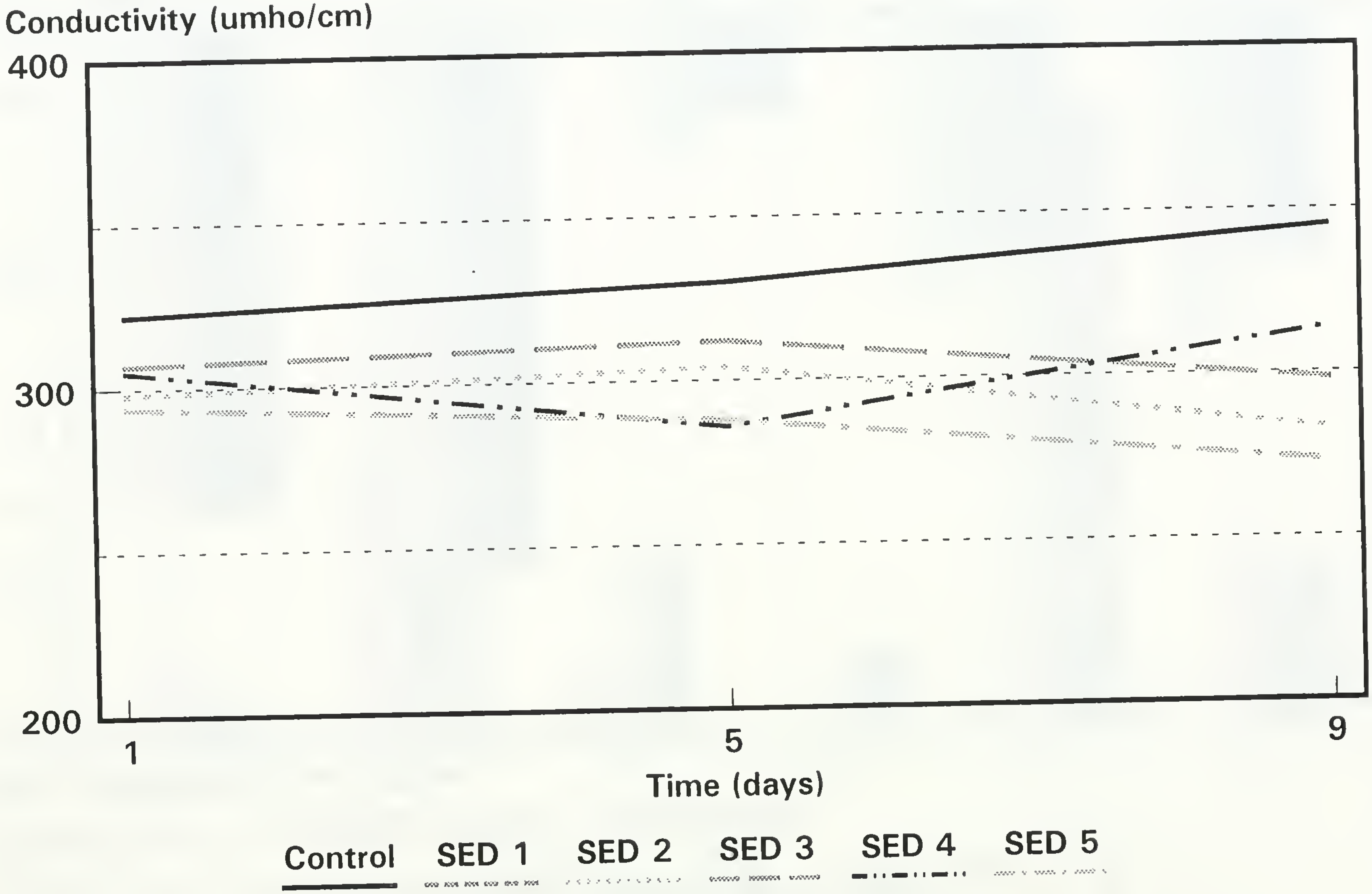
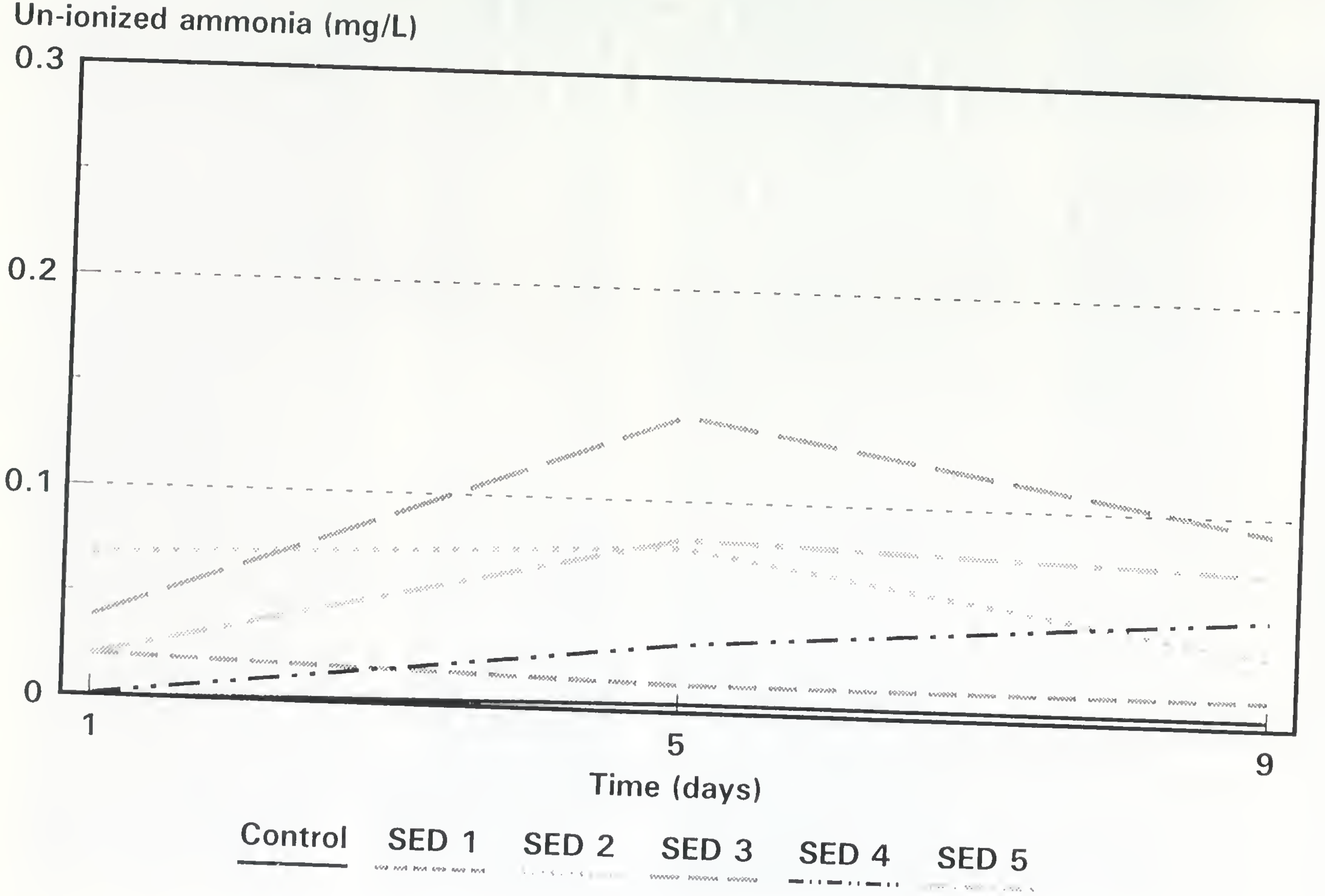


Figure 6: Un-ionized ammonia in 1:4 Exposure



Sed #2, #3 and #4 was approximately 50% lower than those attained in the control animals.

4.0 DISCUSSION

Analysis of variance was carried out to evaluate the main effects of test condition (water:sediment ratio) and sediment type on the two biological endpoints and their interaction. Test condition (water:sediment ratio) did not affect midge survival ($p<0.83$) for the control and five test sediments selected for this study. Significant differences in organism lethality was evident depending on sediment type ($p<0.0001$). Sediment #4 was acutely toxic in both the 1:1.7 and 1:4 experiments. The interaction between sediment type and exposure regime was also not significantly important.

Similarly, the observed significant effects on growth were due to sediment type ($p<0.0001$). Growth was also affected by the test design ($p<0.02$), however, Bonferroni's pairwise comparison of the growth data indicated no significant difference. The main effect interaction term was also not significant ($p<0.34$). Independent examination of the growth results for each test method, indicated a similar ranking of sediment quality using multiple comparison analysis. Differences in biological effects could be attributed to the test sediment and not to the test method. Midge growth in the control sediment attained acceptable average weight of >0.6 mg, dry weight (EC, 1995). As expected, the test sediments resulted in a range of sublethal growth effects. Using either test method, Sediment #2 yielded the smallest organisms and had the greatest impact of midge growth, a moderate growth reduction was measured for Sediment #3 and #5. Spearman Rank Correlation analysis further confirmed the similarity in growth data between the two test procedures ($r=0.94$; $p<0.03$).

Reproducibility of the test results is another useful measure of the accuracy that could be achieved with either test method. This study showed excellent agreement among test precision values (% C.V.) for each test endpoint (Table 3). Even though the mean midge mortality reported for the test sediments was slightly higher than those found in previous sediment toxicity test, the mean, standard deviation and coefficient of variation were remarkably similar for the two test designs. The coefficient of variation matched for the mortality endpoint (36.3% and 36.9%) and for the growth endpoint (21.4% and 19.5%). This suggests that both procedures had an equal test performance following standardized methodology.

Stability in water quality is an important consideration in the development of a standard toxicity test method. Degradation could be a result of the test design or generated from the sediment itself. Overall, pH of the overlying water was relatively stable in both 1:1.7 and 1:4 exposures for all test and control sediment. Mean pH values remained within 1 unit of each other (7.5 to 8.4) and the associated standard deviation was comparable between the two test methods. Dissolved oxygen concentrations were also similar between the two test methods despite the difference in the time aeration commenced, e.g. delayed by 12 hours in the 1:4 exposures. The average conductivity readings were also comparable between the 1:1.7 and 1:4 exposures, for the majority of the sediments. In the Sediment #1 exposures, the first conductivity reading was considerably higher in the 1:1.7 test chambers, than that recorded for the chambers using the 1:4 ratio. The smaller test volume may have resulted in a concentration of ions in the water column, relative to the larger, more diluted water volume. The change in conductivity throughout the experiment was highest in 5 of the 6 sediments used in the 1:1.75 test method.

The greatest variation in overlying water quality parameters occurred for the ammonia concentrations, particularly in the 1:1.7 tests. Loadings appeared to be related to sediment type in some cases. Average un-ionized ammonia readings were an average of 3.3 times higher in the 1:1.7 test containers (0.03 to 0.30 mg/L), for each test sediment as compared with the 1:4 test method (0.01 to 0.09 mg/L). The trend in ammonia measurements also varied according to the test method. For the 1:4 test chambers, ammonia levels reached a maximum half-way through the test and decreased to just above starting concentrations by Day 9. On the other hand, in the 1:1.7 test chambers, for Sediment #3, #4 and #5, ammonia levels did not level off or decline by the time the test was terminated. The pH and test temperature, variables that regulate the dissociation of ammonia, could not explain differences in ammonia concentrations between tests, given the similarity of these parameters for both test methods and among sediment types (Emerson *et al.*, 1975). *C. tentans* has been shown to be sensitive to the un-ionized form of ammonia (NH_3) (Schubauer-Berigan *et al.*, 1995). The increase in un-ionized and total ammonia may be a result of the higher loading capacity of the sediment and faster build-up of metabolic wastes and resulted in a continuing degradation of overlying water quality. Sediment toxicity test methods developed by US EPA (1994) stipulates that water quality should not change by >50%. This criteria was not met for either test method, however, a comparative examination of the data indicate a substantial reduction in the degree of change in un-ionized ammonia for the 1:4 test method.

Treatment	1:1.75 Exposure % Change in un-ionized ammonia	1:4 Exposure % change in un-ionized ammonia
Control	- 61%	+ 21%
Sediment #1	- 25%	- 37%
Sediment #2	+ 126%	- 51%
Sediment #3	+ 1282%	+ 142%
Sediment #4	+ 320%	+ 32%
Sediment #5	+ 162%	+ 268%

It is difficult to determine whether the differences in water quality had any effect on midge survival and growth, given the lack of duplicate measurements. Given the degree in similarity in the relative responses among treatments for the two test designs, it is unlikely that water quality characteristics played a significant role. The effects observed were similar between the different sediment to water ratios and would be a result of sediment contaminant concentrations. Also the interaction term of sediment type and test method, which both could contribute to the overlying water quality, was found not to be statistically important for either endpoint. In the case of ammonia toxicity, the un-ionized ammonia concentration found in this study were well below acute lethal concentrations reported for *C. tentans*. Schubauer-Berigan *et al.*, (1995) reported a water-borne, 10-day LC50 of 4.6 mg/L NH_3 at a pH of 7.8, using midge larvae of a similar age as those used in this study. Employing an acute:chronic ratio of 20 would yield a value of 0.2 mg/L. At these levels it is possible that sublethal effects could be elicited. This concentration was exceeded for at least two of the test sediments using the smaller sample volume.

In addition, the study examined two techniques for measuring organism growth. Differences in growth affects was evaluated by either (1) using individual, wet (or fresh) weights, or (2) using an average individual biomass that was calculated by dividing the pooled dry weight by the total number of animals surviving in each test chamber. Differences in growth among sediment types were virtually identical between the two approaches. The benefit of using the former approach is that it allows for within-replicate examination of the data and is not affected by unequal organism survival. It also permits the animal tissue to be analyzed for chemical body burdens or preserved for additional parameters e.g. body length, head capsule width (Norberg-King *et al*, 1994). The downside of this approach is it tends to be time-consuming and needs to be completed in a consistent manner to ensure a minimal change in body weight due to evaporation. It was quite evident that the wet weight determinations were highly reproducible in this study. The advantage of using dry weights for monitoring growth is that the samples could be dealt with over a longer time periods, providing greater flexibility. The negatives include the need for a sensitive, more expensive balance, samples are prone to subtle changes in weight gain, the data are subjected to differential survival rates and limits any further examination of the test organisms.

5.0 CONCLUSIONS

1. The 1:1.7 and 1:4 (sediment:water ratio) exposures resulted in identical toxicity ranking using *C. tentans* in a 10-day test. Significant relationship was found between the biological endpoints between the two tests. This was based on the outcome using five different field sediments. This suggests either test method could provide appropriate and meaningful sediment evaluations for a variety of contaminated sediments.

2. Test reliability and reproducibility was consistent between the two test methods supporting interchangeability. This occurred despite a number of inherent differences in the two test methods e.g. test chamber size, number of replicates, number of organisms per replicate, and the quality and quantity of food.

3. Individual wet weight measurements or pooled dry weight determinations could be used for interpreting sublethal growth effects. This provides a choice that would better accommodate the specific needs, test objectives, and technical capability of a laboratory.

4. To minimize changes in overlying water quality, a larger test volume could be a more appropriate method under static conditions. Parameters such as ammonia and conductivity are subject to change during the test and should be monitored frequently.

Alternately, a daily static-renewal approach using the smaller test volume would improve and maintain a more stable overlying water quality. Maintaining acceptable and stable water quality becomes an increasingly important consideration in longer-term toxicity tests.

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